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The *Cyclamen graecum* group, how many species?

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Cyclamen graecum is a well-defined evolutionary unit that separated from other *Cyclamen* species about 10 million years ago (Yesson & Culham 2006; Yesson, Toomey & Culham, 2009). It is genetically isolated and there are no records of it hybridizing naturally with other species. However, over that time it has begun to form separate populations that themselves might later become species. These are currently recognised at the subspecies level (Mathew 2013) and have distinct geographic distributions (Figure 1) showing isolation by water between populations. *C. graecum* subsp. *graecum* occupies mainland Greece and nearby islands, *C. graecum* subsp. *candicum* occurs in Crete and C. *graecum* subsp. *anatolicum* is found in Cyprus, Rhodes and areas of Turkey near the Mediterranean coast. However, life with plants would be far too easy if the story were that simple, there are records of *C. graecum* subsp. *graecum* from the western end of Crete leaving it in close proximity to *C. graecum* subsp. *candicum* with the potential to hybridise. And the story is not even that simple.

We were provided with 23 samples of *C. graecum* for analysis by the Cyclamen Society originating from expeditions and private collections (Table 1). These were labelled as nine *C. graecum* subsp. *graecum*, eight *C. graecum* subsp. *candicum* and six C. *graecum* subsp. *anatolicum*. The samples include plants from all the main distributional areas for each subspecies. We extracted and sequenced the DNA following the methods previously described (Könyves and Culham 2014a). Using DNA sequences from six different regions of chloroplast DNA totalling 6182 base pairs of DNA sequence per sample (with a small number of missing data for three samples only). The DNA sequences were aligned with each other so that we could work out the degree of match and degree of difference between samples. The differences were counted by computer to generate a map showing the DNA similarities and the number of changes from sample to sample as described in Könyves and Culham (2014b). This pattern of variation is represented by a simple table that shows counts of the number of differences in DNA sequence between each pair of samples (Table 2) and a diagram (Figure 1) showing the steps between each sample on which we have coloured the circles based on the field identification of the species and shaded areas based on geographic occurrence.

Unlike the study of *C. libanoticum* (this issue) we both expected, and found, a substantial amount of DNA sequence variation. Yesson et al. (2009) previously estimated the evolutionary age of *C. libanoticum* at 1.0-1.4 million years while *C. graecum* has been around for about 10 million years so there has been ten times longer to accumulate changes in DNA sequence. In *C. libanoticum* we found only one base change in the DNA sequence so in *C. graecum* we might easily expect 10 or more and we actually find over 30 changes. Is this plausible? Yes, we argued that *C. libanoticum* has lost some of its genetic diversity as a result of a historically small population size, in contrast the wide distribution of *C. graecum* would support a population that has spread and grown over time.

If the assertion made at the beginning of this article is accepted, and subspecies are geographically separate we would expect to see three very distinct groups of samples, one for each subspecies. However, there are some surprises in the data. Firstly, perhaps a small surprise only, that subsp.

graecum and subsp. *candicum* overlap in their DNA sequence profile (circle H5, Fig. 1). Three samples in H5 are from Crete and one is from the Peloponnese so the genetic overlap coincides with the geographic overlap suggesting there is still gene exchange (hybridization) between the two subspecies. Generally the recognition of something at subspecies level suggests incomplete separation but the beginnings of a drifting apart that might later become a full separation.

Perhaps the biggest surprise, is sample C74 (Table 1) which was gathered in Rhodes. Literature suggests (Mathew 2013; Grey-Wilson 2002) that all *C. graecum* on Rhodes is subsp. *anatolicum* and the field identification was based on this assertion. However the field notes say "Leaves and flowers resemble subsp. *candicum*". The DNA sequence shows us very clearly that this sample belongs to *C. graecum* subsp. *candicum* and it matched three samples from Crete! This is a very good illustration of the need to identify the plant from its individual features rather than from its geographic origin. It does beg the question – how did it get there, and that is not something we can attempt to answer here.

Also very notable was the high degree of genetic separation between C. *graecum* subsp. *anatolicum* and the other two subspecies. This separation is represented by at least 13 changes in DNA sequence (known as 'base substitutions').

Over all, two of the three subspecies of *C. graecum* show genetic overlap that coincides with geographic overlap but the third C. *graecum* subsp. *anatolicum* is genetically distinct showing a large number of DNA changes from the other two subspecies, and it must be a candidate for recognition at the species level.

The split between *C. graecum* subsp.   and *C. graecum* subsp. *anatolicum*, at 2.9-3.4mya, is older than the average speciation age of 2.3my for the genus *Cyclamen* (Yesson, Toomey & Culham, 2009), so it would be entirely consistent to treat *C. graecum* subsp. *anatolicum* as a species rather than a subspecies.

Hildebrand's name *Cyclamen maritimum* (Hildebrand, 1908, p291) is the earliest name published at species level for what is currently called *C. graecum* subsp. *anatolicum* so no new names are required and no new status required for any names. Therefore we propose that the *C. graecum* group now comprises two species, *C. graecum* and *C. maritimum*. *C. graecum* retains only two subspecies, *C. graecum* subsp. *graecum* and *C. graecum* subsp. *candicum* (Table 3). This would be consistent with species concepts elsewhere in the genus *Cyclamen* and properly reflect the genetic and geographic isolation of this element of the group.

Table 3. Proposed classification of the *C. graecum* group

Current name in use	**Proposed name in use**
C. graecum subsp. *graecum*	*C. graecum* subsp. *graecum*
C. graecum subsp. *candicum*	*C. graecum* subsp. *candicum*
C. graecum subsp. *anatolicum*	*C. maritimum*

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Table of samples from which DNA was extracted for *Cyclamen* research				
Reading Code	**List Name**	**Collector**	**Provenance**	**DNA group**
C3	*C. graecum* subsp. *graecum*	Tile Barn 'B'	Prostos; Peloponnese	H5
C4	*C. graecum* subsp. *anatolicum*	Tile Barn 'A'	Monte Smith; Rhodes	H9
C13	*C. graecum* subsp. *candicum*	Tile Barn 'E'	Fourfouras; Crete	H2
C39	*C. graecum* subsp. *candicum*	CSE 96465	Above Askifou; Crete	H5
C63	*C. graecum* subsp. *graecum*	unknown	Rhodopou; Crete	H5
C68	*C. graecum* subsp. *graecum*	CSE93002	site 93/02; Astros; Peloponnese	H6
C69	*C. graecum* subsp. *candicum*	CSE96111	site 96/07; Fourfouras; Crete	H2
C70	*C. graecum* subsp. *graecum*	unknown	Voula Attica	H6
C71	*C. graecum* subsp. *candicum*	CSE96459	site 96/26; Imbros Gorge; Crete	H3
C72	*C. graecum* subsp. *candicum*	CSE96001	site 96/02; Kouloukanas Mountains; Crete	H2
C73	*C. graecum* subsp. *anatolicum*	unknown	Near Liveras; Cape Kormakiti; N. Cyprus	H8
C74	*C. graecum* subsp. *anatolicum*	CSE91466	site 91/14; Monte Smith; Rhodes. Leaves and flowers resemble subsp. *candicum*	H2
C75	*C. graecum* subsp. *anatolicum*	CSE91313	site 91/14; Monte Smith; Rhodes	H9
C76	*C. graecum* subsp. *graecum* f. *album*	CSE96566	site 96/38; Rhodopou; Crete	H5
C77	*C. graecum* subsp. *graecum*	unknown	Thalassa Limonari; Meganisi	H7
C78	*C. graecum* subsp. *graecum* f. *album*	R. & E. Frank	Gythion; Peloponnese; type specimen	H7
C79	*C. graecum* subsp. *graecum*	CSE93642	site 93/23; Hydra	H7
C80	*C. graecum* subsp. *graecum*	CSE93644	site 93/03; Poros	H6
CK121	*C. graecum* subsp. *anatolicum*	CSE09120T	site 09/11; Alanya; Turkey	H9
CK139	*C. graecum* subsp. *candicum*	unknown	Platanias; Crete	H1
CK140	*C. graecum* subsp. *candicum*	unknown	Above Askifou; Crete	H3

CK141	*C. graecum* subsp. *candicum*	unknown	Malaxa; Crete	H4
CK142	*C. graecum* subsp. *anatolicum*	CSE08422T	site 08/32T; W of Taşucu; Turkey	H9

	C3	C4	C13	C39	C63	C68	C69	C70	C71	C72	C73	C74	C75	C76	C77	C78	C79	C80	CK121	CK139	CK140	CK141	CK142
C3																							
C4	17																						
C13	7	18																					
C39	0	17	7																				
C63	0	17	7	0																			
C68	4	17	7	4	4																		
C69	7	18	0	7	7	7																	
C70	4	17	7	4	4	0	7																
C71	6	19	9	6	6	9	9	9															
C72	7	18	0	7	7	7	0	7	9														
C73	15	6	16	15	15	15	16	15	17	16													
C74	7	18	0	7	7	7	0	7	9	0	16												
C75	17	0	18	17	17	17	18	17	19	18	6	18											
C76	0	17	7	0	0	4	7	4	6	7	15	7	17										
C77	3	16	6	3	3	3	6	3	5	6	14	6	16	3									
C78	3	16	6	3	3	3	6	3	5	6	14	6	16	3	0								
C79	3	16	6	3	3	3	6	3	5	6	14	6	16	3	0	0							
C80	4	17	7	4	4	0	7	0	6	7	15	7	17	4	3	3	3						
CK121	17	0	18	17	17	17	18	17	19	18	6	18	0	17	16	16	16	17					
CK139	2	15	5	2	2	2	5	2	4	5	13	5	15	2	1	1	1	2	15				
CK140	6	19	9	6	6	6	9	6	0	9	17	9	19	6	5	5	5	6	19	4			
CK141	4	17	7	4	4	4	7	4	6	7	15	7	17	4	3	3	3	4	17	0	6		
CK142	17	0	18	17	17	17	18	17	19	18	6	18	0	17	16	16	16	17	0	17	19	17	

Table 2. Number of pairwise DNA substitutions.

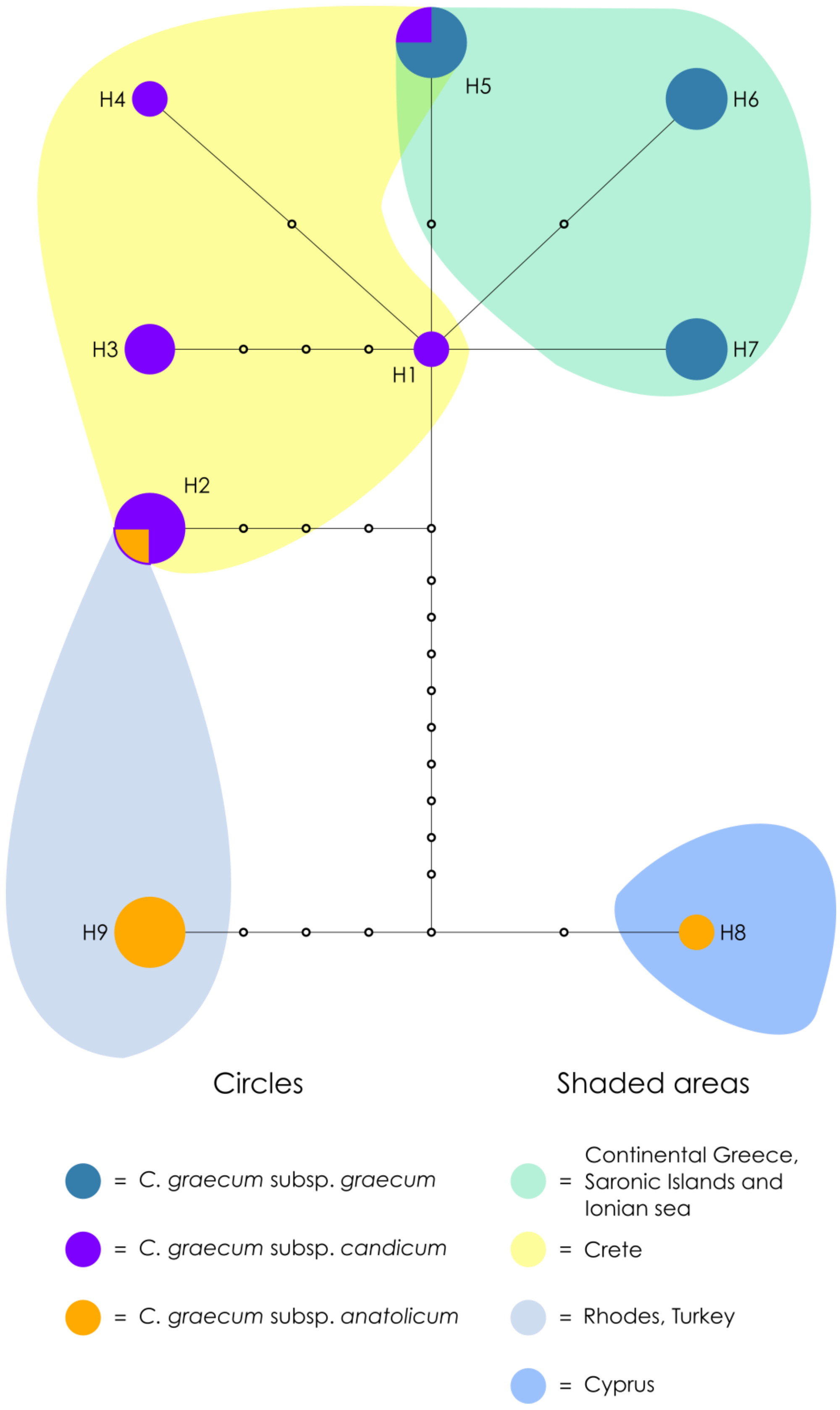

Figure 2. Haplotype network of *C. graecum* samples based on six chloroplast DNA regions. Open circles indicate 1bp change and length of line does not have a meaning. Coloured circles are recorded DNA sequence types. Haplotype numbers (H) correspond to those found in Table 1. Shaded areas show geographic groupings.

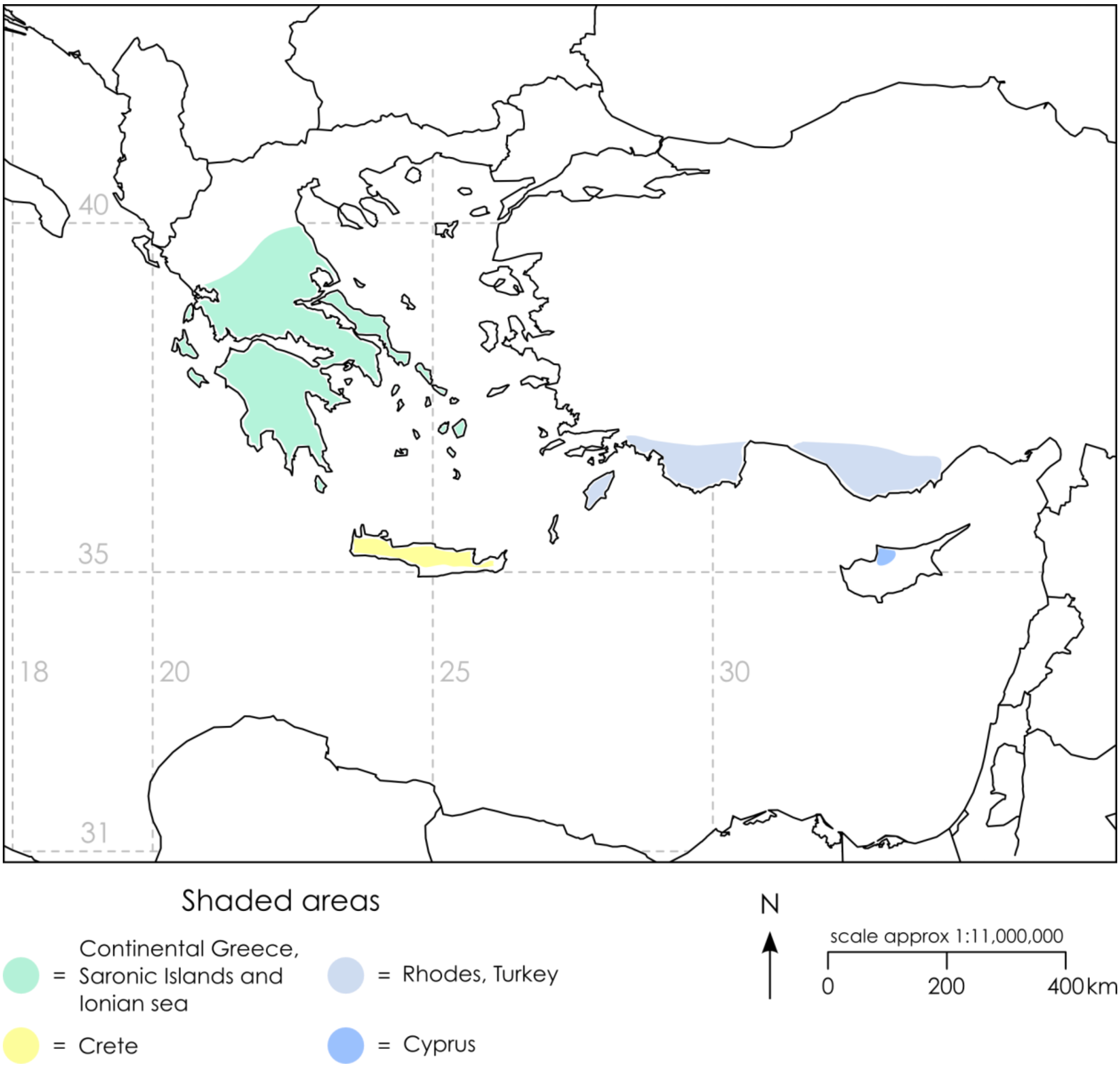

Figure 1. Geographic distribution of *C. graecum*. Shaded areas illustrate geographic groupings corresponding to Figure 2.